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Mitochondrial DNA from neurospora crassa. Structure and genetic function

Terpstra, Peter

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S U M M A R Y

The mitochondrial DNA (mtDNA) from the ascomycete *Neurospora crassa* consists of double stranded covalently closed circular molecules, with a homogeneous length of 19 µm, as has been demonstrated by others.

We have investigated the mtDNA of wildtype strain E 5297. Mitochondria of this strain contain a population of circular DNA molecules heterogeneous in length and smaller than the 19 µm circular DNA, which is also present in strain 5297. The buoyant density in CsCl of a covalently closed circular DNA fraction, isolated from mitochondria of strain 5297, is mitochondrial. This suggests that the small heterogeneous circular DNA could be derived from the 19 µm circular DNA. This relationship, however, could not be investigated, because the isolation of the small circular DNA was not reproducible (Chapter 3).

mtDNA from *Neurospora crassa* strain E 5256 (19 µm circles) was isolated on preparative scale as a collection of linear fragments. With equilibrium density centrifugation in CsCl, sedimentation analysis and denaturation-renaturation techniques it could be established that the distribution of GC basepairs in the mtDNA molecule is heterogeneous. A continuous stretch with a length of at least one third of the molecule has a 6% lower GC content than the 40% average GC content of the whole molecule (Chapter 4).

Cleavage of the mtDNA with restriction endonucleases yields a collection of specific fragments, which can be separated according to size by gel electrophoresis. Endonuclease Bam HI, Eco RI and Hind III cleave the DNA in 5, 12 and more than 30 fragments respectively. Analysis of partial cleavage products allowed us to determine the sequence of the 5 Bam HI and 12 Eco RI fragments. The 5 Bam HI recognition sites could be located on the Eco RI fragments by isolation of the individual Eco RI fragments and redigestion with endonuclease Bam HI. In this way two overlapping cleavage maps could be constructed. In addition we determined the position of the largest Hind III fragment on the map (Chapter 5).

The cleavage map of the mtDNA provided a framework for the localization of genes. From DNA-RNA hybridization experiments we conclude that the mtDNA contains one gene for the large (24S) and one for the small (17S) mitochondrial ribosomal RNA. The genes are found in the region of the mtDNA, relatively rich in GC, on two neighbouring fragments. Their mutual distance is about 900 basepairs. This confirms the existence of a ribosomal RNA precursor, which has been found previously
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which has been found previously by others. The place of the 24S RNA on the cleavage map provided a starting point for ordering 8 Hind III fragments within the largest Eco RI fragment (Chapter 6).

Hybridization of mitochondrial transfer RNAs (tRNA) to mtDNA restriction fragments leads to the conclusion that about 80% of the tRNA genes can be found in a limited area, of maximally 15% genome length. This area is next to the 24S gene. The other tRNA genes are scattered over the whole genome. Some preliminary experiments suggest that in the area of the ribosomal RNA genes, also a gene for a ribosomal 5S RNA can be found (Chapter 7).